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Relationship of CD86 surface marker expression and cytotoxicity on dendritic cells exposed to chemical allergen

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Abstract

Human peripheral blood-derived dendritic cells (DC) respond to a variety of chemical allergens by up-regulating expression of the co-stimulatory molecule CD86. It has been postulated that this measure might provide the basis for an in vitro alternative approach for the identification of skin sensitizing chemicals. We recently reported that DC, exposed in culture to the highest non-cytotoxic concentrations of various chemical allergens, displayed marginal up-regulation of membrane CD86 expression; the interpretation being that such changes were insufficiently sensitive for the purposes of hazard identification. For the work presented here, immature DC were derived from human monocytes and treated with the chemical allergens 2,4-dinitrobenzenesulfonic acid (DNBS), nickel sulfate ($NiSO_4$), p-phenylenediamine (PPD), Bandrowski's base (BB), hydroquinone (HQ) and propyl gallate (PG) for 48 h at concentrations which induced both no to slight to moderate cytotoxicity. For comparison, DC were treated with the irritants sodium dodecyl sulfate (SDS), benzoic acid (BA), and benzalkonium chloride (BZC) at concentrations resulting in comparable levels of cytotoxicity. CD86 expression, as measured by flow cytometry, was consistently up-regulated (ranging from 162 to 386% control) on DC treated with concentrations of chemical allergens that induced approximately 10–15% cytotoxicity. The irritants BA and BZC did not induce up-regulation of CD86 expression when tested at concentrations that induced similar levels of cytotoxicity. SDS, however, up-regulated CD86 expression to 125–138% of control in 2/4 preparations when tested at concentrations which induced similar toxicity. Our results confirm that chemical allergens up-regulate CD86 expression on blood-derived DC and illustrate further that up-regulation of CD86 surface marker expression is more robust when DC are treated with concentrations of chemical allergen that induce slight to moderate cytotoxicity.

Keywords: Chemical allergen; Contact sensitization; In vitro alternatives; Dendritic cells

Introduction

Langerhans cells (LC) play an important role in the induction phase of contact allergy. After contact with a chemical allergen, LC migrate via the afferent lymphatics from skin to draining lymph nodes. During this migration, maturation takes place and the cells change phenotypically by up-regulating expression of the adhesion molecule CD54 and co-stimulatory molecules CD80 and CD86 (Coutant et al., 1999; Cumberbatch et al., 1996). Studies on contact allergy have been limited because LC are a minor cell population within the epidermis and are difficult to isolate in abundance. As a result, investigators have devised culture conditions to generate dendritic cells (DC) in large numbers from peripheral blood monocytes and these cells are routinely used as LC surrogates (Chapuis et al., 1997; Sallusto and Lanzavecchia, 1994; Schuler et al., 1995; Steckel et al., 1995). Several investigators have proposed measuring changes in cell surface marker expression on DC following chemical allergen treatment as an in vitro model for contact sensitization. Aiba et al. (1997, 2000) generated

Abbreviations: DC, dendritic cell; GM-CSF, granulocyte macrophage colony stimulating factor; IL-4, interleukin-4; TNFα, tumor necrosis factor-alpha; DNBS, dinitrobenzenesulfonic acid; BA, benzoic acid; PPD, p-paraphenylenediamine; SDS, sodium dodecyl sulfate; $NiSO_4$, nickel sulfate; BB, Bandrowski's base; HQ, hydroquinone; PG, propyl gallate; BZC, benzalkonium chloride.

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DC from peripheral blood monocytes, exposed them to various chemical allergens and irritants for 24 h, and reported changes in surface marker expression (CD86, CD54, HLA-DR) with select chemical allergens. In a similar model, Degwert et al. (1997) measured surface marker expression on DC after chemical allergen treatment and suggested this measure could be used to discriminate chemical allergens from irritants. Coutant et al. (1999) illustrated that monocyte-derived DC expressed higher surface marker expression of HLA-DR, CD86, CD40, and CD54 in the presence of haptens (aminophenol, chlorpromazine hydrochloride, DNCB, nickel sulfate) as compared to irritants (SDS, benzoic acid). Increases greater than 200% of control in the mean fluorescence intensity (MFI) of CD86 were observed in DC exposed to sub-toxic concentrations of the allergens. Using Langerhans cell-like DC generated from CD34+ cord blood cells, Rougier et al. (2000) demonstrated that incubation with chemical allergen for 48 h induced DC maturation, based on phenotypic changes including CD83, HLA-DR, and CD86. In addition, they showed that non-toxic doses of the weaker allergens (citronellal and coumarin) mostly induced changes in CD86 and not HLA-DR or CD83. For coumarin-treated DC, the number of CD86 cells positive increased in four out of five donors and ranged from 105 to 155% of control cells. Citronellal also induced increases in four out of five donors with the increase in CD86-positive cells ranging from 121 to 212% of control. Finally, Tuschl and Kovac (2001), in a similar model using Langerhans cells and immature DC from peripheral mononuclear blood cells, showed increases in cell surface expression of CD86, CD54, and HLA-DR antigens after chemical allergen treatment, again while irritants had no effect on these markers. Increases in the MFI of CD86 expression in DC treated with the highest non-cytotoxic concentration of all allergens tested were reported. The magnitude of positive responses ranged from approximately 120% of control induced by cinnamaldehyde to approximately 160% of control induced by trinitrobenzene sulfonic acid.

We recently reported our efforts to measure changes in surface marker (HLA-DR, CD54, CD80, CD86) expression on DC exposed to the highest non-cytotoxic concentration of chemical allergens and concluded that the system, under the conditions described therein, did not provide enough sensitivity for an in vitro method with sufficient dynamic range for assessing the contact sensitization potential of a chemical (Hulette et al., 2002). Specifically, no allergen-induced increases in CD86 expression were observed; the Mean Fluorescence Intensity values for the treated cells never exceeded that of the control DC. Based on our experience and what has been reported by others in the literature, it appears that treatment with non-cytotoxic concentrations of contact allergens most often induces only modest (less than 200% of control) up-regulation in the expression of CD86.

For this report, we turned our attention to the range of concentrations of chemical allergen that induced slight to moderate cytotoxicity and focused on CD86 expression. For comparison, we explored the effects of chemical irritants on CD86 expression when treated at concentrations that induced a decrease in cell viability to approximately 85 to 90%.

Methods

Chemicals. 2,4, dinitrobenzenesulfonic acid (DNBS, 99%), sodium dodecyl sulfate (SDS, 99%), nickel sulfate ($NiSO_4$, 99.9%), benzoic acid (BA, 99%), and propyl gallate (PG, 98$^+$%) were purchased from Aldrich Chemical Co (Milwaukee, WI). p-Phenylenediamine (PPD, 98%), benzalkonium chloride (BZC), and hydroquinone (HQ, 99.9%) were purchased from Sigma Chemical Company (St. Louis, MO). Bandrowski's base (BB, >98%) was purchased from ICN Biomedicals Inc. (Aurora, OH). Tumor necrosis factor-α (TNFα), granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) were purchased from R and D Systems (Minneapolis, MN). DNBS, SDS, $NiSO_4$, BA, PPD, BZC, and HQ were formulated in complete media. Stock concentrations of PG and BB were initially dissolved in dimethyl sulphoxide (DMSO, Sigma Chemical Co). TNFα, GM-CSF, and IL-4 were reconstituted from lyophilized stocks in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA, Sigma Chemical Company). Stock concentrations of chemical allergen were sterile filtered through a low protein binding 0.2 μm Acrodisc Syringe Filter (Gelman Laboratory, Ann Arbor, MI). Subsequent dilutions were performed in complete media. Final in-well concentrations of DMSO were less than 0.1%.

Generation of immature DC from peripheral blood leukocytes. Leukocytes were purchased from Pennsylvania Plasma (North Brunswick, NJ). The preparation was diluted with equal parts RPMI-1640 containing 10% heat inactivated fetal bovine serum (Hyclone Laboratories, Logan, UT), L-glutamine (2 mM, Gibco BRL), penicillin G sodium (50 units/ml, Gibco BRL), streptomycin sulfate (50 μg/ml, Gibco BRL), and 2-mercaptoethanol (55 μM, Gibco BRL, Grand Island, NY), hereafter referred to as complete medium. The diluted leukocytes were separated over Ficoll-Paque (Amersham Pharmacia Biotech, Uppsala, Sweden) and the mononuclear fraction was collected and washed three times in complete medium. The cells were counted using a Coulter Counter (Beckman-Coulter Inc., Miami, FL) and the concentration adjusted to 5×10^6/ml. 30 ml of the cell suspension was plated in a T75 flask, corresponding to 2×10^6 cells/cm^2, and the flask was incubated for 2 h at 37 °C/5% CO_2. At the end of the incubation period, the non-adherent cells were removed and the flask was gently washed with complete medium to remove any residual non-adherent cells. 10 ml complete